MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

July–September 2020—Volume 135, pp. 491–500

https://doi.org/10.5248/135.491

Notes on rust fungi in China 8. *Pucciniastrum tiliae* life cycle and new host plants inferred from phylogenetic evidence

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Abstract—The life cycle connection between spermogonial and aecial stages of a rust fungus found on *Abies holophylla* and uredinial and telial stages on *Tilia mongolica* and *T. mandshurica* collected in northeast China were confirmed by phylogenetic analyses. The rust, identified as *Pucciniastrum tiliae*, was confirmed by morphological observations. The life cycle of this rust fungus is reported for the first time in China, and *A. holophylla* and *T. mongolica* represent new host plants for the species.

Key words—phylogeny, *Pinaceae*, *Pucciniastreae*, *Tiliaceae*, tree disease

Introduction

Pucciniastrum tiliae was first described with uredinial and telial stages on *Tilia cordata* var. *japonica* [≡ *T. japonica; Tiliaceae*] collected in Hokkaido, Japan (Hiratsuka 1897). This species has been since reported on *T. amurensis* in Korea, China, and Russian Far East; *T. americana* and *T. kiusiana* in Japan; *T. mandshurica* in China and Russian Far East; *T. maximowicziana* [= *T. miyabei*] in China and Japan; *T. platyphyllos* in Japan; and *T. tuan* in China (Hiratsuka 1936, 1958; Ito 1938; Cummins 1950; Cummins & Ling 1950; Wang 1951; Kim 1963; Tai 1979; Hiratsuka & al. 1992; Azbukina 2005,

2015). The life cycle of this fungus was revealed by Kamei in Japan in 1934 through inoculations of basidiospores from teliospores on *T. japonica* and *T. maximowicziana* into needles of *Abies sachalinensis* var. *mayriana* (*Pinaceae*) (Hiratsuka & al. 1992). Although *P. tiliae* is distributed in northeast China (Tai 1979), there are no reports of a spermogonial and aecial host in the country.

During field investigation of rust fungi in northeast China, we collected uredinial and telial stages of *Pucciniastrum* on *T. mandshurica* and *T. mongolica* in the mountains of Jilin and Heilongjiang Provinces. We also observed occurrences of aecia producing long peridia on *Abies holophylla* near infected trees of *Tilia*. Therefore, we suspected life cycle connections between the rust on *Abies* and *Tilia*. Inoculation of plants by spores are an appropriate method to clarify rust life cycles (Ji & al. 2017a, b). However, collection and propagation of plants from the mountains of Maple Valley (Jilin) and Fenix National Park (Heilongjiang) where we collected specimens are strictly prohibited as they are nature conservation areas. Therefore, we applied molecular analyses to resolve these problems and to clarify the life cycle (Liu & Hambleton 2013, Ji & al. 2016, Padamsee & McKenzie 2017, Scholler & al. 2019). We report here the results of phylogenetic analyses and morphological observations of the specimens.

Materials & methods

Molecular analyses

Uredinial and telial stages on *Tilia mandshurica* and *T. mongolica* collected in mountains in Jilin and Heilongjiang Provinces, China, were used for molecular analyses. The spermogonial and aecial stages on *Abies holophylla* collected in Jiaohe, Jilin Province, China, were also used for analyses.

The total genomic DNA was directly extracted from c. 200 spores obtained from single sori on the leaf of each specimen using similar methods reported by Ji & al. (2016, 2019). Specimens used in the experiments were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU); all data sequenced in this experiment were deposited in GenBank as shown in specimens examined.

The sequences obtained from specimens were aligned following Ji & al. (2019). ITS and 28S sequences related to *P. tiliae* were retrieved from GenBank and added to phylogenetic analyses (Ji & al. 2019). The accession numbers of these data are shown in the phylogenetic trees (Figs 1, 2). Phylogenetic trees were constructed using *Melampsora laricis-populina* Kleb. as outgroup according to Ji & al. (2019). The alignment and trees were deposited in TreeBASE under http://purl.org/phylo/treebase/phylows/study/TB2:S24909 (Figs 1, 2).

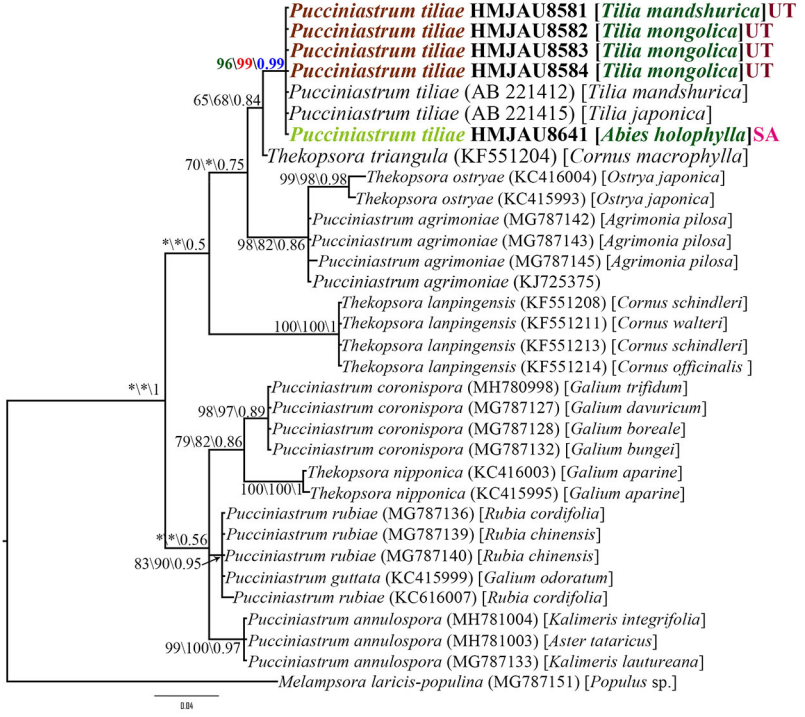

Fig. 1. Phylogenetic tree constructed by Bayesian method based 28S rDNA sequences. MP/ML bootstrap values precede Bayesian posterior probabilities (Bpp) on the nodes. Asterisks (*) indicate bootstrap values <50% or Bpp <0.5. Sample data include species name, (voucher specimen or GenBank accession number), and [host plant]. Sequences generated in this study are shown in bold face. **SA**= Spermogonial and aecial stages; **UT** = Uredinial and telial stages.

Morphological observations

Light (LM) and scanning electron (SEM) microscopy were used to examine morphological characters of rust specimens, including the size and shape of sori and spores following Ji & al. (2019).

Phylogenetic results

The 28S dataset comprised 33 sequences with 496 total characters, of which 394 were constant, 30 parsimony-uninformative variable, and 72 parsimony-informative. Parsimony analysis yielded one parsimonious tree with TL = 156, CI = 0.7692, RI = 0.9231, and RC = 0.7101. Bayesian analysis resulted in average standard deviation of split frequencies of 0.008983. The ITS dataset comprised 39 sequences of 745 total characters, of which 228 were parsimony-informative. Parsimony

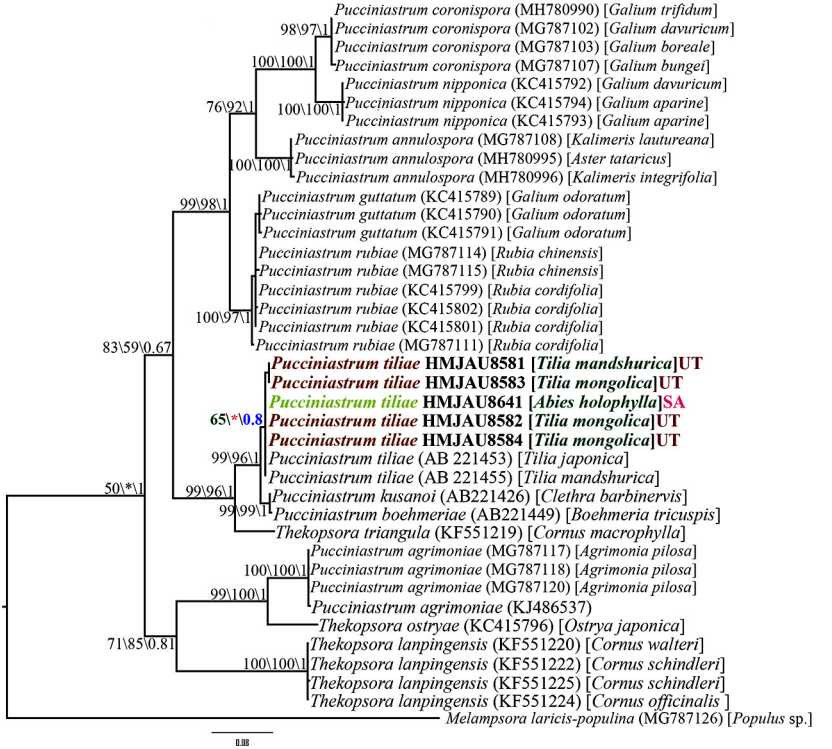

Fig. 2. Phylogenetic tree constructed by Bayesian method based on ITS rDNA sequences. MP/ML bootstrap values precede Bayesian posterior probabilities (Bpp) on the nodes. Asterisks (*) indicate bootstrap values <50% or Bpp <0.5. Sample data include species name, (voucher specimen or GenBank accession number), and [host plant]. Sequences generated in this study are shown in bold face. **SA**= Spermogonial and aecial stages; **UT** = Uredinial and telial stages.

analysis yielded one parsimonious tree with TL = 543, CI = 0.8066, RI = 0.9417, and RC = 0.7596. Bayesian analysis resulted in average standard deviation of split frequencies of 0.003112. Tree topologies formed by MP, ML, and MCMC methods were identical among trees. The Bayesian phylogenetic trees are shown in Fig. 1 (28S) and Fig. 2 (ITS).

Both 28S and ITS phylogenetic trees included the uredinial and telial stages on *T. mandshurica* (HMJAU 8581) and *T. mongolica* (HMJAU 8582, 8583, 8584) and the spermogonial and aecial stages on *A. holophylla* (HMJAU 8641) in a monophyletic clade (Figs 1, 2). Genetically identical to one another, they can be considered the same species. The life connection between spermogonial and aecial stages on *A. holophylla* and uredinial and telial stages on *T. mandshurica* and *T. mongolica* is therefore strongly supported by the phylogenetic analyses.

Taxonomy

The phylogenetic analyses reveal that this rust fungus has a heteromacrocyclic life cycle, producing spermogonia and aecia on *Abies holophylla* (Fig. 3D) and uredinia and telia on *Tilia mandshurica* and *T. mongolica* (Figs 3A–C). LM and SEM observations showed that the morphological characteristics of spermogonial and aecial specimens on *A. holophylla* collected from the field were identical with the descriptions of *Pucciniastrum tiliae* reported by Hiratsuka (1936) and Hiratsuka & al. (1992), except for outer wall surface structure of the peridial cells (Figs 3D, 4A–C, A–D). Under SEM, these walls are minutely verrucose, in contrast to the smooth surface described by Hiratsuka & al. (1992). Previously, *P. tiliae* spermogonial and aecial stages have been reported on *Abies* species only from Japan, so this represents the first report of the *P. tiliae* life cycle from China. *Abies holophylla* is a new spermogonial and aecial host plant and occurs in northeastern China, Korea, and Russian Far East.

After clarifying the life cycle connection of *P. tiliae* with phylogenetic analyses, we described its morphology based on specimens collected in the field.

The morphological characters of uredinial and telial specimens on *T. mandshurica* and *T. mongolica* were similar to one another (Figs 3A–C, 4D–F, 5E,F) and identical to the descriptions of *P. tiliae* by Hiratsuka (1936), Hiratsuka & al. (1992) and Azbukina (2005, 2015). No researcher has previously reported *P. tiliae* on *T. mongolica* (Hiratsuka 1936, 1958; Kim 1963; Tai 1979; Hiratsuka & al. 1992; Azbukina 2005, 2015), so we add *T. mongolica* (known from Mongolia, northern China, and eastern Russia) as a new host for the rust.

Pucciniastrum tiliae Miyabe, Bot. Mag. Tokyo 11: 47, 1897. Figs 3–5

Spermogonia amphigenous, pale yellow to yellowish, subcuticular, type 3 (Cummins & Hiratsuka 2003). Aecia with long peridia, *Peridermium*-type, hypophyllous, cylindrical, orange-yellow. Peridia colorless, rupturing at the sides and apex; peridial cells ovate to ellipsoid, slightly overlapping, inner and outer walls thin, minutely verrucose. Aeciospores globose to ellipsoid, 15.0–22.0 × 13.0–16.5 μm (av. = 18.5 × 15.0 μm), walls hyaline, 1.0–2.0 μm (av. = 1.5 μm) thick, densely verrucose with annulate verrucae.

Uredinia hypophyllous, scattered to gregarious, subepidermal, small, round, covered by the epidermis, rupturing with pores, pulverulent. Peridia hemispherical, delicate, firm, rupturing at the apex, peridial cells small,

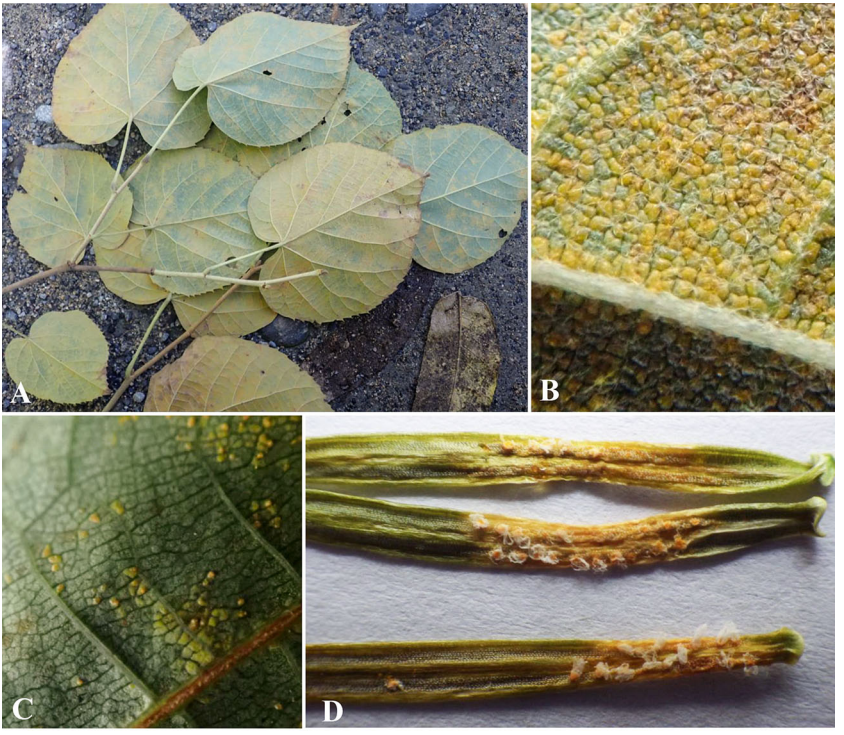

Fig. 3. *Pucciniastrum tiliae.* A. Uredinia and telia on lower leaf surface of *Tilia mongolica.* B. Telia produced on lower leaf surface of *T. mongolica.* C. Uredinia produced on lower leaf surface of *T. mongolica.* D. Aecia with long peridia produced on needles of *Abies holophylla.*

irregularly polygonal, walls smooth, thin, ostiolar cells rounded, walls smooth. Urediniospores subglobose, ellipsoid, obovate, or oblong, 16.5–25.5 × 8.0–15.5 µm (av. = 20.5 × 12.5 µm), walls echinulate, 0.5–2.0 µm (av. = 1 µm) thick. Telia hypophyllous, subepidermal, scattered or in small groups, at first orange-yellow, then brown to reddish in color. Teliospores forming under the epidermis, attached laterally, subglobose, oblong, or somewhat angular, divided into 2–6 cells by vertical or oblique septa, 21.0–37.0 × 5.5–14.0 µm (av. = 30.5 × 9.5 µm), walls thin, 0.5–1.0 µm (av. = 0.7 µm) thick, smooth.

Specimens examined—**CHINA, Jilin Province, Jiaohe**, stages 0 & I, on *Abies holophylla* Maxim., 16 June 2019 (HMJAU8641, GenBank MN294706, MG787116); stages II & III, on *Tilia mongolica* Maxim., 11 September 2017 (HMJAU8583, GenBank MG787149, MG787124; HMJAU8584, GenBank MG787150, MG787125); **Helongjiang**

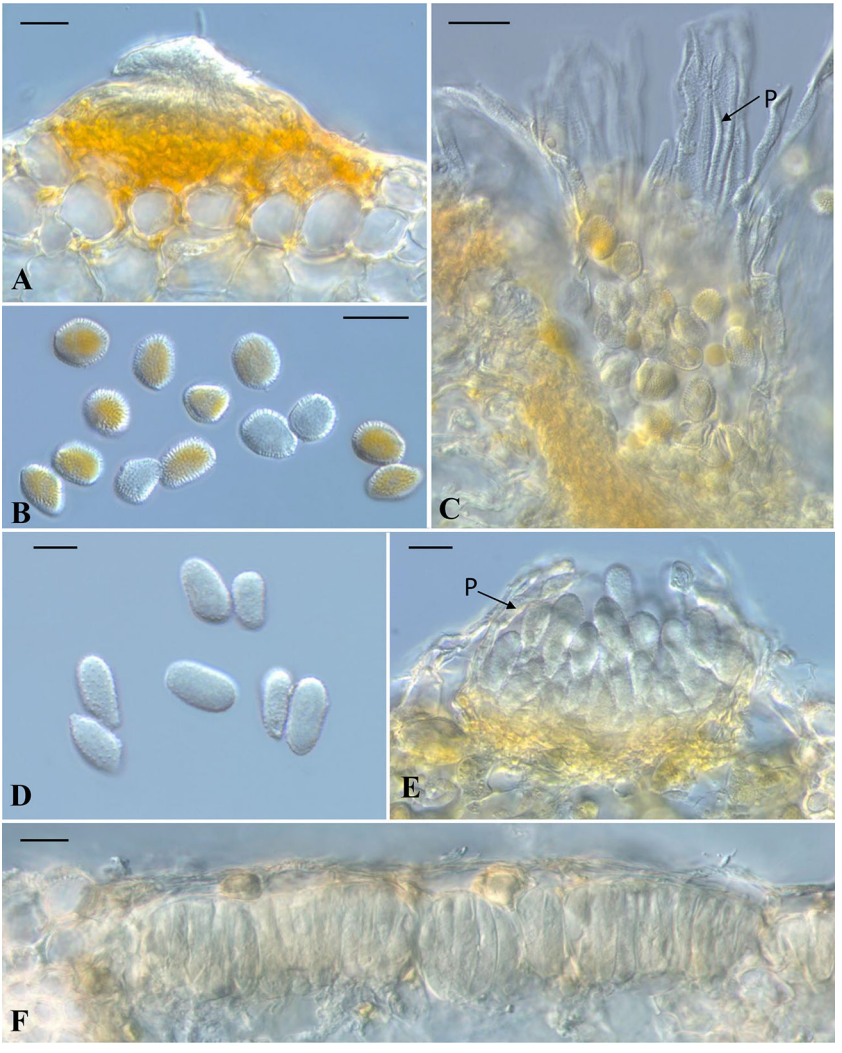

Fig. 4. *Pucciniastrum tiliae* observed with LM. A. Vertical section of spermogonium (type 3). B. Aeciospores with verrucose surface. C. Vertical section of aecium surround by peridium (P). D. Urediniospores with echinulate surface. E. Vertical section of uredinium with peridium (P), opening by central pore. F. Vertical section of telia with laterally attached teliospores, covered by host epidermis. Scale bars: A, C = 20 µm; B = 30 µm; D, E = 10 µm; F = 15 µm.

Province, Wuchang, stages II & III, on *T. mongolica*, 9 September 2017 (HMJAU8582, GenBank MG787148, MG787123); stages II & III, on *T. mandshurica* Rupr. & Maxim., 9 September 2017 (HMJAU8581, GenBank MG787147, MG787122).

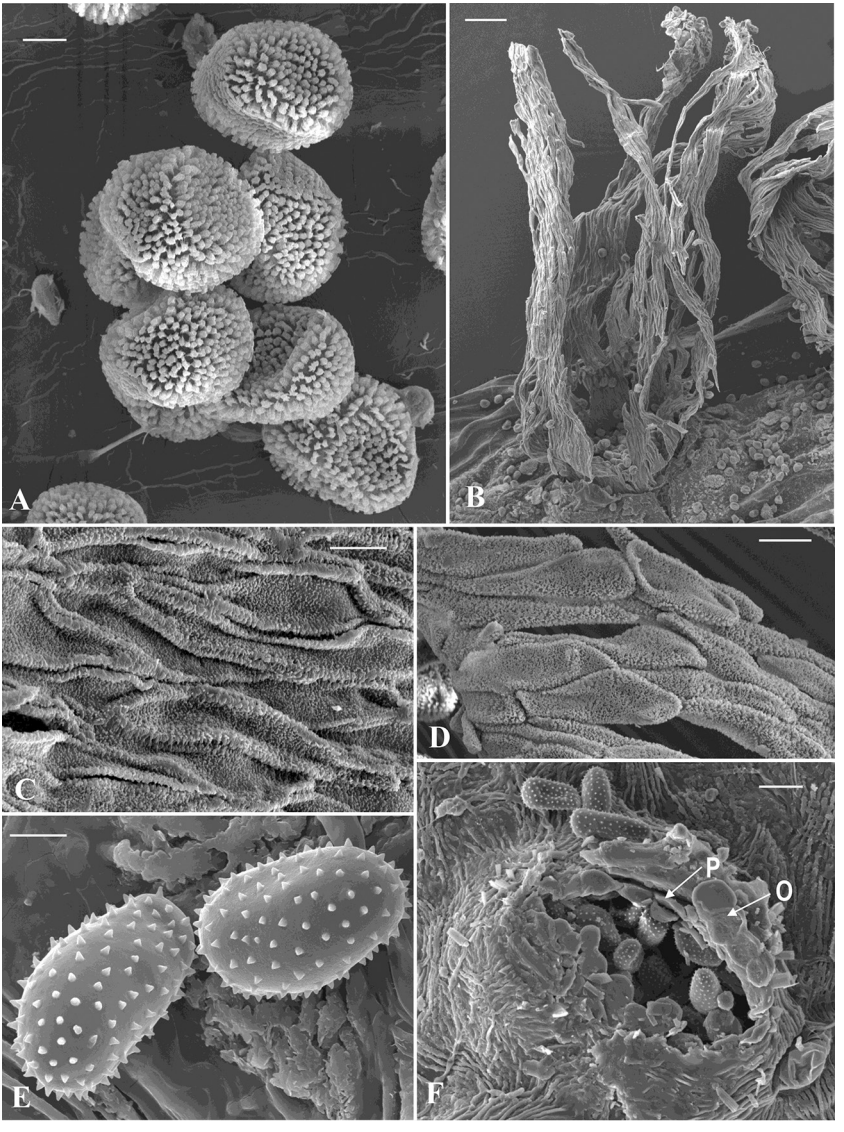

Fig. 5. *Pucciniastrum tiliae* observed with SEM. A. Aeciospores with densely verrucose surface. B. Aecium with long peridium. C. Outer walls of peridial cells. D. Inner walls of peridial cells. E. Urediniospores with echinulate surface. F. Uredinium with peridium (P) and ostiolar cells (O), opening by central pore. Scale bars: A, E = 5 μm; B = 100 μm; C, D = 20 μm; F = 10 μm.

Hosts & distribution within China—***Abies holophylla***, Jilin (new host; this paper). ***Tilia amurensis*** Rupr., Jilin (Ito 1938, Tai 1979). ***Tilia mandshurica***, Heilongjiang (Ito 1938, Hiratsuka 1958, Tai 1979). ***Tilia mongolica***, Heilongjiang, Jilin (new host; this paper). ***Tilia tuan*** Szyszył., Guizhou (Hiratsuka 1958, Tai 1979).

Acknowledgments

This work was financed by the Fungal Flora in Jilin Province (20130206073NY). We thank Dr. E.H.C. McKenzie (Manaaki Whenua Landcare Research, Auckland, New Zealand) and Prof. C.M. Denchev (Bulgarian Academy of Sciences, Sofia, Bulgaria) for critical reading of the manuscript and suggestions.

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